Red Blood Cell Metab

I. Properties of RBCs
   A. Provide a reducing compartment in within oxidizing blood
   B. Can reg. O_2 binding by controlling effectors
   C. 120 day camping trip– no macromolec. Synth, or metab (beyond glycolysis and PPP)
   D. Biochem of RBCs
      i. Specialized for O2 and CO2 transp., carry only the essentials
         a) Carries only food and first aid – glycolysis and PPP
      ii. Derived from pluripotent stem cells
         a) Hypoxia --> HF-1 (hypoxia inducible transcr. Factor) -->erythropoietin
         b) Erythropoietin (EPO)
            - synth'd in kidney peritubular cells
            - Stim's growth of RBC precursors
            - Suppresses apoptosis
            - Stimulates globin synthesis
            - Med. Importance – Recombinant EPO treats patients w/decreased RBC synth
               - Patients with end stage kidney disease
               - HIV patients taking certain Rtase inhibitors
               - Cancer patients on certain chemo types
               - Post-surgery
      iii. Shape max's gas and solute exchange – dependent upon cytoskel and membrane struct
      iv. Built to pick up O_2 and drop off CO_2 in lungs – Higher pH & [O_2] (Haldane effect)
      v. Built to pick up CO_2 and drop off O_2 in peripheral tiss– lower pH & [O_2] (Bohr effect)

II. Composition of RBC Membrane

A. Lipids – More Phosphatidic acid, sphingomylein, and cholesterol than other places

i. Cholesterol adds rigidity to the RBC membrane

ii. uneven distribution on leaflets

a) inner - more phophatidyl<u>ethanolamine</u> and phosphatidyl<u>serine</u>

b) outer - more phophatidyl<u>choline</u> and sphingomyelin

B. Proteins -

i. asymmetical orientation

a) outside freq. Glycosylated e.g. Glycophorin

b) membr spanning portion w/series of alpha helices w/hydrophobic side chains

c) intracellular or extracellular domains may be exposed to solvent

ii. types

a) intrinsic – big chunks w/in membrane interior –don't wash off easily (e.g. Band 3)

b) extrinsic – assoc with, but not embedded in membr - easily dissociated (e.g Spectrin)

C. Specific Key Intrinsic Proteins

i. Band 3 (a.k.a. Anion Exchange Protein AE1)

a) Transmemb glycoprotein with 12 membrane spanning helices

b) Forms channel for electroneutral Cl^- and HCO_3^- exchange through membrane

c) Closely related siblings in liver (AE2) and brain/heart/muscle (AE3)

d) N Term interacts w/ankyrin

e) associates with glycolytic enzymes, e.g. G3P DH

ii. Glycophorins – glycoprotein w/heavily glyc'd outside, 1 alpha helix spans membrane

a) Sialic acid in glycocalyx gives neg charge to outside of RBC, preventing adherence

b) Receptors for malarial parasites

c) Determinants of blood grps

- H substance forms by linkage of GDP-Fuc to gal-glcNAc-R
- H substance gets either GalNAc or Gal tacked on by GalNAc/Gal transferase
  - Fuc-Gal-GlcNaC-R-**GalNAc** = A substance (A blood type)
  - Fuc-Gal-GlcNaC-R-**Gal**= B substance (B blood type)

Blood Type	Antigens	Serum Ab's
A	A	Anti B
B	B	Anti A
AB	AB	None (Universal donor)
O	None	Anti A and B (Univ. Recip)

D. Role of cytoskeleton – allows deformation + resilience

i. RBCs must pass through capillaries

a) Spectrin – alpha and beta subunit, antiparallel coiled-coil, triple helical repeats

- bridge b/n membrane and cytoskel
- Links Band 3 (via anykyrin) and Glycophorin C (via actin and Band 4.1)

E. Membrane defects

i. Hereditary spherocytosis – Defects in interaction with Band 3 (beta/alpha spectrin, ankyrin, and occasionally Band 3)

a) result in reduced interaction b/n cytoskeleton and membrane--->less resilience

b) cells are deformed, cleared by spleen ---> splenomegaly

ii. Hereditary elliptocytosis, pyropoikilocytosis

a) mutations in alpha or beta subunits of spectrin mess up tetramer formation

b) cells take elliptical or oval shape b/c or horizontal interaction of spectrin

c) One band 3 mutation can cause ovalocytosis, too

III. Energy Metab in RBCs – to power pumps and prevent injury

A. Glycolysis
   i. generates ATP – energy for Na+/K+ pump and Ca2+ pump
   ii. 2,3-BPG -
   iii. NADH – reduces metHB (Fe3+) to Hb (Fe2+)

B. PPP
   i. generates NADPH to reduce
      a) metHB (Fe3+) to Hb (Fe2+)
      b) Reduced Glutathione (GSSG) to Reduced Glutathione (2GSH)

C. GSH – Glutathione
   i. Roles
      a) detox of H_20_2
      b) Reduction of Oxidized protein thiols (Protein-S-R $\xrightarrow{2GSH}$ Protein-SH + R
      c) non enzymatic reduction of Met Hb to Hb – 12% of all MetHb reduction
   ii. Synthesis
      a) ATP + Glu +Cys $\xrightarrow{\text{glutamyl cystein sythetase}}$ gamma glutamyl cysteine + ADP + Pi
      b) gamma glutamyl cysteine + glycine $\xrightarrow{\text{GLUTATHIONE SYTHETASE}}$ glutathione
   iii. Regeneration
      a) GSSG is reduced to 2GSH by NADPH and glutathione reductase
      b) 2GSH is oxidized to GSSG by H_2O_2 and glutathione peroxidase

D. 2,3-BPG
   i. Roles
      a) promotes release of 0_2
      b) Prevents excess accum. of ATP
      c) Reg's own synth by neg feedback inhib
   ii. Synth and regeneration
      a) 1,3-BPG from glycolysis $\xrightarrow{\text{BPG Mutase}}$ 2,3-BPG (neg feedback inhib)
      b) 2,3-BPG $\xrightarrow{\text{2,3-BPG PHOSPHATASE}}$ 3-Phosphoglycerate (--->glycolysis)
   iii. Influences on 2,3-BPG
      a) <u>Hypoxia</u>
         - Heart failure, acclimation to high altitude, etc. cause increase in 2,3 BPG
      b) <u>Genetic conditions</u> alter the binding isotherm of Hb
         - Hexokinase deficiency – less glycolysis, less 2,3 BPG – shifts to left
         - Pyruvate kinase deficiency – less PEP--->Pyr, more 2,3 BPG – shifts to right
      c) Altering the binding isotherm of Hb
         - <u>Pathological conditions</u> - Anemia, Obstructive pulmonary disease, cystic fibrosis, congenital heat disease & hyperthyroidism
         - <u>Other conditions</u> - high altitude, and exercise
         - <u>Fetal Hb</u> binds O2 w/greater affinity, and 2,3 BPG w/lower affinity

IV. Oxidation of Hb
  A. MetHb ($Fe3^+$) - oxidized form of Hb
    i. Iron inevitably oxidizes from $Fe2^+$ to $Fe3^+$, and won't bind O_2
      a) Spontaneously
      b) Drugs or toxic oxidants, e.g. components of tobacco smoke
    ii. Reduction
      a) *MetHb Redutase I (a.k.a. Cytochrome B5 reductase aka diapherase I) *primary PW*
        - NADH $\xrightarrow{\text{CYT B5 REDUCTASE}}$ Reduces 2MetHb to 2Hb
      b) MetHb Reductase II (a.k.a. Diapherase II) – *Minor PW ~ 10% MetHb*
        - MetHb + NADPH $\xrightarrow{\text{DIAPHORASE II}}$ Hb + $NADP^+$
      c) Glutathione (2GSH +MetHb --> GSSG + Hb) *Minor PW ~ 12% MetHb*
    iii. MetHb Anemia
      a) MetHb Reductase II defect – mild – induced by oxidant stress
      b) Cyt B5 Reductase defect – mild to severe, depending on the mutation
      c) Cyt B5 Reductase deficiency – causes reduced enzyme concentration – usually mild
      d) Hb M (*M*ultiple types) – mutations *in Hb subunits* make iron more prone to oxidation
  B. Coping with oxidative damage to Hb (Antioxidants)
    i. <u>Superoxide dismutase</u> – eliminates superoxide, the highly reactive oxygen species
    ii. <u>Ascorbate</u> -
    iii. <u>Vitamin E</u> – Reacts with oxidants forming peroxyvitamin E---> reduced to Ascorbate
  C. Oxidation related disorders
    i. G6P DH Deficiency –
      a) Enzyme <50% effective - affects PPP –> oxidative damage to RBCs
      b) X-linked -common in people of tropical African/Asian/Mediterranean descent
      c) Patients have increased sensitivity to oxidative stress
      d) Common medical complications
        - Neonatal jaundice – can result in neurological damage
        - Acute Hemolytic Anemia – life threatening in children
        - Hemolytic attack, induced by a variety of drugs, chemicals, or diseases causes:
          - lipid oxidation (damages RBC membrane)
          - Destruction of spectrin (damages RBC cytoskeleton)
          - Oxidation of Hb (produces more MetHb)
          - Production of H_2O_2 – causes Lactate buildup

V. Solute transport across the RBC membrane
  A. Transporters act like enzymes
    i. facilitating an energetically unfavorable process
      a) ions must shed hydration shell, pass hydrocarbon layer, and rehydrate
    ii. providing a solute specific path
  B. Not everything needs a transporter
    i. O_2 and CO_2
  C. Needed for ions and specific polar solutes
    i. Na^+, K^+, H^+, HCO_3^-, Ca^{2+}
    ii. Glucose, H_20
  D. Types of transport
    i. Uniport – single solute transported via facilitated diffusion (e.g. Glut1)
    ii. Symport – Cotransport of two solutes in the same direction
    iii. Antiport – Cotransport of two solutes in opposite directions (Band 3 aka AE1)

VI. Key transporters of the RBC

A. Band 3

i. Roles

a) antiport of Cl^- in and HCO_3^- out (by Carbonic anhydrase)
- essential for efficient transport of CO_2

b) Binds membrane and cytoskeleton

ii. How it fills its roles - Protein binding capactiy

a) N term inside cell (unusual)
- G3PD, PGK, Aldolase, Ankyrin, 4.1, Hb, Hemochromes

b) C term binds Carbonic anhydrase

c) Ankyrin and 4.1 bind other places as well

iii. Action of specific bound proteins

a) Hb/hemichrome – Band 3's role in senescence (aging) of RBCs
- oxidative damage to proteins and lipids marks RBC's for destruction by spleen
- One type of damage is the partial unfolding of Hb to hemichrome
  - Hb binds weakly to N term of Band 3
  - Hemichrome binds stongly to N term of Band 3
    - aggregates form, and generate and epitope
    - epitope recognized on cell surface by IgG
    - RBC is cleared by macrophages

b) Carbonic Anhydrase – catalyzes hydration of CO_2
- In periph tissue
  - Incoming CO_2 + cytosolic H_2O $\xrightarrow{\text{CARBONIC ANHYDRASE}}$ H_2CO_3
  - H_2CO_3 loses HCO_3 to Band 3 antiport, and H^+ remains
  - Haldane effect – (deoxygenation of Hb increases its ability to carry CO_2)
    - H^+ and cytosolic HbO_2 yield HHb and free O_2 that leaves the RBC
    - HHb picks up CO_2 from tissue and forms Carbam Hb
- In lungs- reverse of what happens in tissue
  - Haldane effect - (Oxygenation of Hb decreases its ability to carry CO_2)
    - O_2 serves as an affector in the release of CO_2 from Hb
      - Carbam Hb releases CO_2, forming HHb
  - HHb combines with O_2, forming HbO_2 and free H^+
  - H^+ combines with H_2CO_3 forming H_20 and CO_2
  - CO_2 is released from the RBC

B. GLUT 1 – facilitated uniport glucose transport

i. Intrinsic membrane protein with 12 membr spanning domains

C. Aquaporin (AQP1) – abundant facilitated uniport H_20 transport

i. Consists of a tetramer of polypeptides, each subunit has 6 membr sapnning alpha helices

ii. Permits single file transport of water, but NOT protons

D. Na^+/K^+ ATPase – Active antiport maintains a concentration gradient

i. 3 Na^+ come into the cytosol, while 2 K^+ go out to the blood